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ORIGINAL PAPER

Cytomorphology of G*entiana kurroo*: an important endangered bitter plant of temperate Himalaya.

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Abstract: *Gentiana kurroo,* a potent bitter drug plant of Indian subcontinent, is under threat due to over exploitation and destruction of natural habitat. We studied the morphophenology and chromosomes of *G. kurroo* on both wild and field grown plants, which is very important for proper identification, conservation and domestication. Results reveal that *G. kurroo* is a perennial herb, and its shoot is represented by flowering branches only. Stem is modified to rhizome. The older rhizomes split into four parts at collar region appearing to fuse together at the ends and is an important diagnostic feature for crude raw materials. Two types of leaves i.e. radical leaves at the base of the plant and cauline leaves on flowering shoot are present. Flowering occurs during September to October with 1-9 inflorescences per plant. Inflorescence is terminal monocasial cysome type. Flowers are protrandus. Anthesis starts around 7.30 am and continued till 10.0 am. Ovary is bicarpillary syncarpous unilocular. Fruit is Capsule and takes 18–20 days to mature after fertilization. Seeds are very small elliptical and 1 000 seeds weigh to 0.1275 g. Chromosomal studies made by usual squash method reveals the species is a genomic allotetraploid with n = 13. The anaphase-I segregation was normal and in none of the cells at Anaphase-I or Telophase-I could any abnormality like laggards, bridges, micronuclei etc. be observed.

Keywords: cytology; morphology; allotetraploid; *Gentiana kurroo*; floral character

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Introduction

Gentiana kurroo Royle (Gentianaceae) is a critically endangered temperate bitter herb of Indian subcontinent region. It is perennial and commonly grows in Kashmir, Himachal Pradesh and adjoining hills of North-Western Himalaya between 1500–3000 m altitudes (Chaudhary and Wadhwa 1984). Commonly known karu/Indian gentian, though not mentioned by Sanskrit writers in Materia Medica (Dymock 1890), is a significant drug of ayurveda (Shahi 1993).

The rhizome and roots of this herb contains some of the most known bitter compounds like gentianine (a crystalline monoterpene alkaloid), amaroswerin (Secoiridoid glycoside), gentianic acid, pectin, and uncrystallizable sugar (Anonymous 1953 and Singh 2008). Gentianine possesses anti-inflammatory, analgesic, anticonvulsant, hypotensive, antipsychotic, sedative, diuretic, antimalarial, antiamoebic and antibacterial properties and amaroswerin gastro-protective (Singh 2008). In Indian system of medicine the root stock is valued as bitter tonic, antiperiodic, expectorant, antibilious, astringent, stomachic, anthelmintic, blood purifier, laxative, depurative, emmenagogue, galactopurifier, febrifuge and carminative (Chopra *et al*. 1956 and Anonymous 1956). It is also medicated for curing skin diseases, leucoderma, leprosy, bronchial asthma, dyppepsia, flatulence, colic, anorexia, helminthiosis, inflammations, amenorrhoea, dysmenorrhoea, strangury, haemorrhoids, constipation and urinary infections (Warrier et al. 1995). The drug is very helpful in removing all kinds of debility and exhaustion of body from prolonged illness, improves digestive system and lack of appetite. The root is used as an ingredient in preparing feed for fattening horses (Qureshi 2007).

The dried roots and rhizomes that are official in Indian pharmaceutical codex is a substitute for true gentian obtained from *Gentiana lutea,* a native of Europe and Asia Minor (Dutt 1928, Dey and Bahadur 1973). Although the species is in high demand but commercial cultivation is absent. Unscientific and heavy extraction from its natural habitat coupled with lack of proper replenishment has made the species endangered. Hence, the Min-

istry of Commerce, Government of India has put it in the negative list of exports vide Notification no. 2 (RE-98) 1997–2002 dated 13 April 1998. Perusal of literature reveals that, detailed information regarding morphophenology scanty. As far as karyotype is concerned no reports were found elsewhere. Different authors studied karyotype of genera rather than kurroo (karu) under the family gentianaceae and reported the chrosome numbers (Federov 1974, Stebbins 1971). The objective of this communication was to provide morphological and cytological data for further utilization.

Material and methods

Morphophnological studies were carried out on both wild (Population P1 near Sirmour, Himachal Pradesh at an altitude 1685 m. m.s.l) and field grown plants (Population P2 in the medicinal garden of college of forestry near Shilley at an altitude of 1550 m. m.s.l). Observations regarding habit, type of root, rhizome, shoot, stem, leaf, flower etc. were compared and recorded as per the description given by Lawrence (1951), Weberling (1989) and Kaufman et al. (1989) for each parameter. All parameters in morphological observations were statistically analyzed for mean & standard error and based on 25 randomly selected plants of same age.

For male meiotic studies, young flower buds were collected from its natural habitat, washed thoroughly and fixed in Carnoy's fixative (absolute alcohol, glacial acetic acid and chloroform in 1:1:1 proportion) for 24 h. Later on they were washed under running tap water to remove all traces of fixative. The fixed material was stored in 70% absolute alcohol. Slides are prepared by the usual squash method (freshly prepared one per cent acetocarmine was used as stainer) and observed under light microscope. For studying the details of chromosomes, 20 cells were marked randomly at the well-spread metaphase stage.

Results

Morphological and floral character

Plants of *Gentiana kurroo* are perennial herbs. The aerial part is mostly composed of radical leaves which occur in a rosette (Fig.1. A). The shoot is represented by flowering branches only which bears cauline leaves (Fig.1. B). The height of the plant is measured as length of flowering shoot and is 17.44 cm long. At full bloom stage, average number of flowering shoots per plant is observed to be 5.19 with a range of one to nine. Under natural conditions, plants of this species mostly occur on southern sites of exposed slopes, between 1 650 to 2 000 m at m.s.l. and are surrounded by grasses. Detailed quantitative features of leaf, shoot and flower are presented in Table 1.

Table 1. Quantitative morphological parameters of *Gentiana kurroo*

Morphological parameters		Dimension/number (Average ± standard error)	Morphological parameters		Dimension/number (Average ± standard error)
Plant height		17.44±1.16 cm	Corolla	No. of petals	5
Root	Length	8.47±1.23 cm		Total length	4.25±0.53 cm
	Diameter	2.13±0.57 mm		Plicae length	1.02±0.11 cm
Rhizome	Length	10.47±1.01 cm	Androecium	No. of Stamens	5
	Diameter	1.75±0.49 cm		Stamen length	2.85±0.39 cm
Leaves	No. of radical	36.50±3.77		Anther size	4.48±1.29 mm
	No. of cauline	22.69±2.74		Pollen size	31.0±1.89 µm
	Length of radical	11.36±0.18cm by 10.94±0.62 mm	Gynoecium	Carpels No.	2
	Length of cauline	6.75±0.12 cm by 4.41±0.22 mm		Ovary length	4.96± 0.40 cm
Flowering	Length	17.44±1.16 cm		Ovary width	6.07±0.93 mm
shoot	No.of flowering shoots/plant	5.19±0.27		Length of bifid stigma	3.50±0.32 mm
	No.of flowers/inflorescence	4.23±0.54	Fruit	Length	5.47±0.38 cm
	No. of flowers/plant	20.48±1.95		Weight	2.03±0.14 g
Flower	Length	7.19±0.35 cm	Seed	Length	42.28±1.43 µm
	Diameter	2.74±0.47 cm		Breadth	15.00±0.77 µm
	Pedicel length	2.06±0.36 cm		1000 seeds weight	0.1275±0.02 g
Calyx	No. of sepals	5			
	Total length	2.56 0.49 cm			
	Fused length	1.34 0.12 cm			

Rootstock, consisting of rhizome and adventitious roots, is perennial and characterized by stout, more or less thick round rhizome with a number of adventitious roots arising from it. Density of adventitious roots is more towards the collar of the rhizome. The rhizome is dusty white to brown in colour. A peculiar characteristic of this species is the splitting of the older rhizome into four parts. These four parts seem to fuse into a single structure which is triangular or quadrangular in outline. The splitting is generally observed towards the upper portion of the rhizome. The unsplit quadrangular portion of rhizome bears a dark brown

thick covering with a number of dormant buds in vertical rows on each quadrangular/triangular surface. Rhizome surface is vertically wrinkled and terminate in a scaly tuft consisting of bases of leaves and flowering shoots. Average dimension of rhizome and root is given in Table 1.

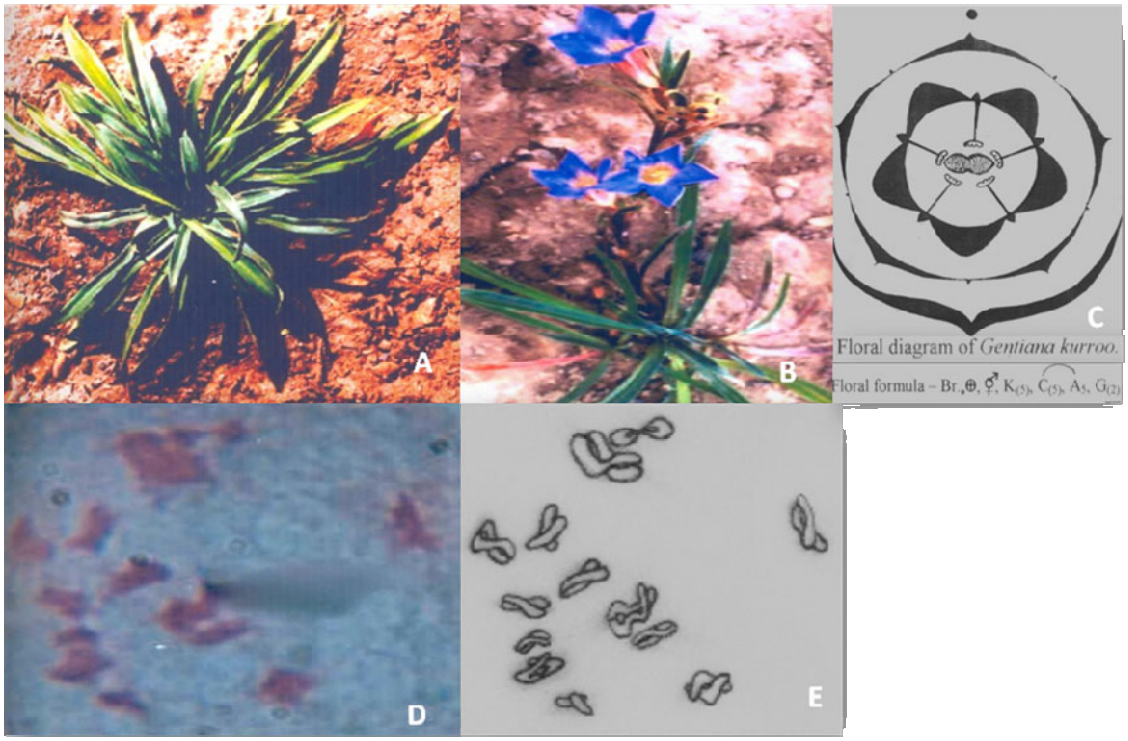

Fig.1 Morphological and floral characters of *Gentiana kurroo*. A—Vegetative stage; B—Flowering stage; C—Floral diagram with floral formula; D—Pollen mother cell at diakinensis (2n=26); E—Diagrametic representation of the bivalents at diakinensis

Stem is condensed and represented by underground rhizome. Aerial stem is represented by decumbent flowering shoot. Two kinds of leaves i.e. radical and cauline are present in this species. Radical leaves are long, narrow, simple, sessile, stipulate, lanceolate, lamina entire, apex acute, leathery in texture, and are covered with thick layer of cuticle. They remain throughout the life cycle of the plant & gradually replaced by newer leaves. Most of the new leaves are developed upon the onset of rainy season. These leaves basally joined in pairs forming a common sheath. The cauline leaves borne on flowering shoots are narrow, linear and in pairs united at base forming a tube around the flowering shoot. These are narrower and smaller as compared to the radical leaves (Table 1) & shed along with the flowering shoot. The venation pattern of leaves is pinnate or unicostate reticulate type. Towards lamina base, the veins are more prominent being thicker.

The arrangement of flowers on the flowering axis is solitary or monochasial cymose (Fig.1. B). Rachis achieves full length 18-23 days after the initiation of flowering shoot from the base of radical leaves, on the tufted annular brown rhizome. Flowers of this species are bracteate, pedicellate, complete (calyx, corolla, androecium and gynoecium), hermaphrodite, actinomorphic, hypogynous and pentamerous. The flowers are large (Table 1) deep violet blue from outside with corolla whitish from inside below the plicae (Fig.1. B). The upper portion of the radiating corolla is also same coloured but spotted with white dots. Flowering occurs between Septembers to October. On average a plant produces 20.48 numbers of flowers (Table 1). Though no nectar gland was observed, bumble bee (*Bombus* sp.), honeybee (*Apis mellifera*) and ladybird beetle (*Coccinella septempunctata*) are observed visiting to flowers of karu. This may be for foraging pollen grains.

Sepals five, gamosepalous, persistent, tubular, lobes linear, more or less equal in size. Tip acute or subulate, retorse. Initially deep green in colour but later changes to warm brown as the capsule matures after fertilization. Odd sepal is posterior to mother axis (Fig.1. C). Calyx lobes 2.56 cm long with the lobes fused upto half or slightly less i.e. 1.34 cm from base. Petals five, gamopetalous, infundibuliform, five lobed limbs, the tips of which are obtuse. The five lobes are characterised by infolding at the fusion lines which are characterized by small intermediate lobes. Petals spotted with white dots around the throat from inside and deep bluish from outside. Corolla base is pale greenish and Corolla tube white coloured from inside. Odd petal is anterior to the mother axis (Fig.1. C). Corolla is 4.25 cm long with plicae about quarter long i.e. 1.02 cm. An important feature of its corolla is that it closes during the night or on dull days opening again at the onset of sun-shine. The calyx and corolla are persistant up to fruit maturation stage, probably to provide protection to the developing fruit. Stamens five, free, epipetalous and alter-

nating with petal lobes. Filament long, fused up to half of the length with the corolla lobe, flattened at base and tapering towards the apex. All stamens are equal in length (Table 1) and introrse. Anthers are white in colour, bicelled, dorsifixed and globose in shape. Anthers are grouped towards the stigma tip in bud condition. As the flowers are about to open and anthers ready to dehisce, the anthers are positioned away from the stigma tip by the change in curvature of the filaments. Anther length is 4.48 mm and dehiscence is longitudinal. The numerous pollen shed from each anther lobe, are more or less round in shape with triporate exine. Average diameter of pollen grain is 31.09 μm. Anthesis begins around 7:30 am and continues up to 10: 00 am.

Bicarpillary, syncarpous, unilocular ovary contains numerous anatropous ovules with parietal placentation. Style is indistinguishable. Ovary occupies central position on the concave thalamus and is superior. The two lobes of stigma remain adpressed together up to anther dehiscence, opening out only after complete anther dehiscence. The stigma tip in adpressed condition remains well below the anther level initially. After anther dehiscence, the stigmatic lobes start opening and the size of carpel increases to push the stigma above the anther level and outside the corolla cavity. The stigma lobes are white in colour which changes to warm brown as the ovary matures after pollination/ fertilization. The open stigmatic lobes are characterized by papillae on the upper surface only which help in trapping pollen grains. Average dimension of carpel is 4.96 cm by 6.07 mm and the bifid stigma is 3.5 mm in length.

Fruit is a capsule with an average length of 5.47 cm. Capsule dehisces longitudinally along the two opened valves beginning from tip exposing numerous seeds which get blown away by wind. Weight of a single freshly harvested fruit is approximately 2.03 mg. Seed is elliptical with one end drawn out like a tail. Seed yield per fruit was observed to be 8.95 mg and that of total seed set per plant (based on an average of 20 flowers per plant) was 167.5 mg. The weight of thousand seeds was observed to be 0.1275 g. The detailed floral and morphological parameters are given in Table 2.

Table 2. Floral and morphological features of *Gentiana kurroo*

Plant part	Characters
Habit and habitat	Temperate and subtemperate herb
Rootstock	Consists f stout parennial rhizome and adventitious roots
Stem	Condensed and represented by underground rhizome. Decumbent stem forms the aerial part
Leaves (1). Radical	Simple, sessil, estipulate, lanceolate, lamina entire and apex acure
(2). Cauline	Narrow, linear, opposite, the bases forming a shealth around the flowering shoot
Inflorescence	Solitary/Monochasial cymose
Flower	Bracteate, pedicellate, complete, hermaphrodite, actinomorphic, hypogynous and pentamerous, Entomophilous (pollinated by bumble bee, honey bee & lady bard beetle), Type of dichogamy Protrandrous, Odour & Necter abscent, Flowering period September – October, Flower opening time 7: 00 am,
(1) Calyx	Sepals five, gamosepalous, persistent, tabular, lobes linear, tip acute, retorse, green in colour, odd sepal is posterior to mother axis
(2) Corolla	Petals five, gamopetalous, Infundibuliform five lobed limb. Odd petal anterior to mother axis, Plicae blue, throat dotted with white dots, base white from inside
(3) Androrcium	Stamens five, haplostamenous, epipetalous, alternate with petal lobes, introrse. Anthers bicelled, dorsi fixed, globose, white in colour, Pollen shape Round to oval, triaperturate , Ather dehiscence time 7:30-10:00 am
(4) Gynoecium	Bicarpillary syncarpous unilocular ovary. Ovule anatropous, placentation parietal, style indistinguishable.
Fruit	Capsule and takes 18-20 days to mature after fertilization
Seed	Small, elliptical, one end drawn out like a tail

Chromosome behaviour during male meiosis

In all the pollen mother cells studied, 13 bivalents were observed at diplotene, diakinensis and metaphase-I stage of meiosis (Fig.1. D & E). The bivalents were characterized by interstitial as well as terminal chaismata. There was observed size difference between different bivalents. Invariably one to two bivalents was seen attached to the nucleolus. The anaphase-I segregation was also normal and in none of the cells at Anaphase-I or Telophase-I could any abnormality like laggards, bridges, micronuclei etc. be observed.

Discussion

Morphological studies

The plants of this species are perennial herbs with creeping or deep rooted rootstock with aerial parts represented by basal leaves and flowering shoot only. The stem has become modified into underground rhizome. The radical leaves are leathery in texture, long, narrow and covered with thick layer of cuticle. Seeds are small (42.28 μm × 15.0 μm) and light weight (1000

seeds weigh 0.1275 g), which helps in their easy dispersal by wind. These are the features which helps *Gentian kurroo* to adopt harsh climatic condition of temperate biome (Abrams 1993). An important feature of the rhizome is its splitting into four parts, and these splits seem to fuse together at both ends, enclosed in thick periderm. This is a very important diagnostic feature that can be very helpful in identifying crude drugs.

Inflorescence is always fixed for a particular species which gives the arrangement of its flowers on the flowering axis (Weberling 1989). However, there exists some confusion with regard to the type of inflorescence in *Gentiana kurroo*. Kirtikar and Basu (1935) report racemose inflorescence while as Chittenden (1956) mentions flowers with terminal position meaning cymose type of infloresence. Even the general inflorescence profile of plants of Gentianaceae is dichasial or monochasial of cymose type (Lawrence 1951). During the course of present investigation, the type of inflorescence observed was terminal and monochasial cymose. In view of this, it appears that the record of racemose inflorescence in *Gentiana kurroo* as reported by Kirtikar and Basu (1935) is erroneous.

The type and shape of flower typifies a species as to its breeding system. The Infundibuliform & colour scheme corolla is ideally suitable for cross pollination. Although approximately only four flowers are present per flowering shoot, but the large size of its flowers makes up for the few number of flowers vis-a-vis pollination vectors. The epipetalous stamens are positioned in such a way that the anthers remain grouped in the central cavity of the corolla above the stigma tip initially. After flower opening the anthers move towards the corolla periphery shedding pollen while as the stigmatic lobes start opening. This protrandrous condition favours cross poliination (Raina et al 2003).

According to Cronquist (1968), there are many characters in living forms which can be considered advanced over primitive characters. Based on the classification of primitive and advanced characters given by Cronquist (1968), *Gentiana kurroo* seems to possess the following primitive and advance features, based on morphological characters.

Primitive characters of *Gentiana kurroo* :- Active cambium, long lived, vascular cambium in a cylinder, simple leaves, leaves net veined, flowers perfect, regular, hypogynous and dehiscent fruit.

Advanced characters of *Gentiana kurroo* :- Temperate habit, herbaceous, petals united, stamens differentiated into filament and anther, carpels fully closed, terminal stigma, carpels united, parietal placentation, pollen triaperturate, cotyledons two.

Chromosome behaviour

There is a predominance of species of *Gentiana* with 2n = 26 (22 species) and 2n=36 (20 species) out of 80 species for which diploid chromosome numbers are known (Federov 1974). Based upon these, the presumed base numbers operating in the genus are 5, 7, 9−13, 15, 18, 20−22 and 26. Base numbers above 12 are considered as secondary base numbers having evolved as a result of ascending and descending dysploidy and /or crosses between forms with different base numbers followed by chromosome doubling (Stebbins 1971). The present observation of 2n=26 in *Gentiana kurroo* appears to be based on x=13 which according to Stebbins (1971) is a secondary base number. It can be presumed that this base number (x=13) has arisen as a result of cross between progenitors with different base numbers. One possibility could be of a cross between progenitors with x=7 and 6. Although x=7 has been reported in *Gentiana nivilis* (Federov 1974) but as per the available literature no species with x = 6 has been reported. If *Gentiana kurroo* has evolved as a cross between the species with x=7 and 6, then species with x = 6 should also be present in nature. *Gentiana kurroo* is a perennial herb and can propagate both by vegetative and sexual means. Vegetative propagation bestows an advantage in the sense that the resultant plant as a result of cross between x = 7 and 6 would be able to perpetuate itself in nature. Chromosome doubling in this plant with 2n = 13 (after a natural cross between x = 7 and 6) during the course of evolution, must have ensured setting of fertile seeds as a result of normal meiosis. However, as long as the other wild progenitor with x= 6 is identified and its karyomorphology compared with the progenitor with x = 7 and also with the present *Gentiana kurroo* (with n = 13), nothing final can be said about the evolution of this species. Based upon the chromosome behaviour during meiosis, diploid chromosome number and the presumed base number, *Gentiana kurroo* seems to be a genomic allotetraploid.

References

Abrams MD. 1993. Genotypic and phenotypic variation as stress adaptations in temperate tree species: a review of several case studies. *Tree Physiology*, **14** (7-8-9): 833−842.

Anonymous. 1953. The Indian pharmaceutical codex: Indigenous drugs. New Delhi: CSIR .V. 1: pp. 64−65.

Anonymous. 1956. *The Wealth of India: A dictionary of Indian raw material and industrial products*. New Delhi: Publication and Information Directorate. CSIR .V. 4, pp. 123−125

Chaudhary HJ, Wadhwa BM. 1984. *Flora of Himachal Pradesh:* Botanical Survey of India, New Delhi: Raje Printers.

Chittenden FJ. 1956. *Dictionary of gardening: A practical and scientific encyclopedia of horticulture.* London: Oxford Clarendon Press, V. II, pp. 871−880.

Chopra RN, Nayar SL, Chopra IC. 1956. *Glossory of Indian Medicinal Plants*. New Delhi: CSIR p. 124.

Cronquist A. 1968. *The evolution and classification of flowering plants*. Boston: Houghton mifflin, pp. 143−145.

Dey KL,Bahadur R. 1973. *Gentiana kurroo*: *The Indigenous Drugs of India. Short descriptive notices of the principal medicinal products met within British India*. New Delhi: Pama Printers, p. 140.

Dut, NB. 1928. *Commercial drugs of India*. Thacker: Spink and Company, pp. 183−185.

Dymock W. 1890. *Pharmacographia indica. A history of the principal drugs of vegetable origin met within British India*. New Delhi: Periodical Expert Book Agency, Vol. II, pp. 510−111.

Federov. 1974. *Chromosome numbers of flowering plants*. West Germany: Otto Koeltz Science Publishers, pp. 328−330.

Kaufman PB, Carlson TF, Dayanandan P, Evans ML,Fisher JB, Parks C, Wells JR. 1989. *Plants: their biology and importance*. New York: Hopper and Raw Publishers, pp. 714−730.

Kirtikar KR, Basu BD. 1935. Indian Medicinal Plants (IInd ed.). Dehradun: Bishen Singh Mahendra Pal Singh. Periodicals expert, Vol. III, pp. 1661−1662.

Lawrence HM. 1951. *Taxonomy of vascular plants*. New York: The Macmillan Company, pp. 670−672.

Quereshi RA, Muhammad AG, Gilani SA, Sultan K, Ashraf M. 2007. *Ethno botanical studies of selected medicinalplants of sudhan gali and ganga chotti hills, District Bagh, Azad Kashmir*. *Pak J Bot*, **39**(7): 2275−2283**.**

Raina R, Behera MC, Chand R, Sharma Y. 2003. Reproductive biology of *Gentiana kurroo* Royle. *Current Science*, **85**(5): 667−670.

Shahi VK. 1993. A significant drug of Ayurveda: Vangamaya-katuki. *Sachitra Ayurveda*, **46**(1): 21−27.

Singh A. 2008. Phytochemicals of Gentianaceae: a review of pharmacological poperties. *International Journal of Pharmaceutical Sciences and Nanotechnology*, **1** (1): 33−36.

Stebbins GL. 1971. *Chromosome evalution in higher plants*. London: Edward Arnold, p.61.

Warrier PK, Nambiar VPK, Ramankutty C. 1995. *Indian medicinal plants: a compendium of 500 species.* Madras: rient Longman Pvt. Ltd., Vol. 3, pp. 72−75.

Weberling F. 1989. *Morphology of flowers and inflorenscence*. New York: Cambridge University Press, pp. 1-2, 17−18.